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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
MOORE, WILLIAM W	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 12/04/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/786,009	XU ET AL.	
	Examiner	Art Unit	
	William W. Moore	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 August 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *12-18 and 21-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12-18 and 21-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Response to Amendment

Applicant's Amendment filed August 22, 2003, has been entered and the more specific limitations introduced by the amendments to method claims 12, 22, 25, and 28 requiring a specific thiol reagent, and the more specific requirement for specific linker nucleic acid sequences introduced in the method of claim 17, remove the bases for the rejections of record of claims 12-14, 16-18, 22, and 25-27 under 35 U.S.C. §§102(a), (b), and (e) stated in the Office communication mailed May 23, 2003. New grounds of rejection are stated herein applying prior art to a more specific limitation made in the Amendment filed August 22, 2003, and restating an earlier rejection of record under the first paragraph of 35 U.S.C. §112. This communication is a non-final communication, however, because Applicant was advised in an interview conducted August 15, 2003, concerning a copending application, serial No. 09/249,543, to overcome obviousness-type double patenting rejections in both applications, either over the other, by amending claims of either or both applications to state more specific claim limitations, limitations of the nature subsequently provided in the amendment herein of August 22, 2003, and acting on the suggestion made during that interview may not prejudice applicant.

Claim Objections

Claim 12 is objected to because of the following informalities: The term "thioester" is misspelled in the preamble of claim 12. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. §112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 12-18 and 22-30 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This new ground of rejection addresses the enablement of the use of a "naturally-occurring intein" or an unspecified "intein" in claimed methods. The terms occur at line 6 of claim 12 as currently amended, line 6 of the text of claim 17 as currently amended, line 5 of claim 25 as currently amended, and line 6 of the text of claim 28 as currently amended. Each instance of the term "naturally-occurring intein" or "intein" is present in a recitation of a group of components that includes two other kinds of components - "an intein derivative" and "an intein mutant" - to be used in forming a C-terminal thioester on a first protein where it is cleaved from a carboxyl-proximal intein in the claimed methods so that a peptide, or a second polypeptide, having an amino-terminal cysteine can be subsequently ligated to the first protein. This is essentially the rejection made in the first communication on the merits mailed July 30, 2002, with regard to the limitation required at page 4 therein for intein components of method claims, i.e., "wherein said intein, or derivative or mutant thereof, is incapable of promoting protein splicing at its amino-terminal fusion with said selected protein". The basis for requiring this limitation is set forth at page 5, lines 6-10 and 19-21 of the communication mailed July 30, 2002, and the limitation is still required because claims 12, 17, 25 and 28 do not exclude the presence of a protein binding domain optionally fused to an intein also fused to a selected protein, and because claim 24 implies that claim 22, from which it depends, permits a protein binding domain to be optionally fused to an intein that is also fused to a selected protein.

The basis for this limitation is also found in teachings at page 7 of the specification that the prior art practice of such ligation methods required the use of an intein "modified to undergo thiol inducible cleavage at its N-terminal junction", citing Chong et al.(1997) and the '714 Patent to Comb et al., both of record. The specification then teaches that

"[t]he ligation method of the instant invention . . . use[es] an intein as described [by] Chong et al." The specification further teaches, at page 8, that "an intein whose protein splicing ability has been blocked by mutation is utilized", providing that, "[t]he mutant . . . retain the ability to undergo the N-S shift, allowing thioester formation between itself and an N-terminal protein", and particularly describes mutation of the "C-terminal asparagine of an intein . . . to an alanine" to produce, page 9, a "thiol inducible cleavage element." Example I at pages 11-12 of the specification specifically describes such a modification of a naturally occurring intein.

The specification nowhere indicates that naturally-occurring inteins having a C-terminal asparagine could be used in any of the methods of claims 12-18 and 22-30 where both the selected protein and a protein binding domain are fused to an intein, no matter what the nature of the thiol reagent used to induce cleavage at the amino terminus of the intein, and the prior art and the specification teach that the presence of a C-terminal asparagine is an obligatorily-conserved feature of all naturally occurring inteins because it is essential for intein splicing, hence intein maintenance, in archeotes, prokaryotes, and eukaryotes. Neither the prior art of record nor Applicant's specification can identify any naturally occurring inteins that will support the practice of the claimed methods and teach away from the use of naturally occurring inteins by uniformly teaching that at least one, specific, alteration must be made of a naturally occurring intein in order to permit the formation of a C-terminal thioester on a target protein upon thiol reagent cleavage of a carboxyl-proximal intein thus allowing subsequent ligation of another polypeptide or peptide at the site of the C-terminal thioester.

It is well settled that 35 U.S.C. §112, first paragraph, requires that a disclosure be sufficiently enabling to allow one of skill in the art to practice the invention as claimed without undue experimentation and that unpredictability in an attempt to practice a claimed invention is a significant factor supporting a rejection under 35 U.S.C. §112,

first paragraph, for non-enablement. See, *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (recognizing and applying the "*Forman*" factors). Cf., *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (citing eight factors relevant to analysis of enablement). Applying the "*Forman*" factors discussed in *Wands*, ***supra***, to Applicant's disclosure, it is apparent that:

a) the specification lacks adequate, specific, guidance for using any naturally occurring intein in a claimed method without modifying it by at least the replacement of C-terminal asparagine with another amino acid,

b) the specification lacks working examples wherein any naturally occurring intein is used in a claimed method and instead teaches that altering a naturally occurring intein by at least the replacement of C-terminal asparagine with another amino acid is required for practice of a claimed method,

c) in view of the prior art publications of record herein, the state of the art and level of skill in the art do not support the practice of claimed method without first making at least one specific alteration of the carboxyl terminal asparagine of a naturally occurring intein, and,

d) unpredictability exists in the art where no members of the class of naturally occurring inteins embraced by the scope of the claims have been reported to persist in a cell in order to be recovered and identified unless they possess a C-terminal asparagine, and where the specification and the prior art do not teach how a naturally occurring intein embraced by the scope of the claims can be used in the practice of a claimed method unless it is modified by at least the replacement of C-terminal asparagine with another amino acid.

Thus the scope of the practice of claimed methods with intein components embraced by the phrases, "naturally occurring intein", and "intein" without modification, cannot be considered to be supported by the present specification even if taken in combination with teachings available in the prior art.

Claims 28-30 are further rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This new ground of rejection addresses the enablement of a generic restoration of protein "activity" in methods of claims 28-30. The specification teaches only restoration of enzymatic activity to enzymes and the specification and the prior art of record herein show that native enzymatic activity and native binding activity can only be restored to a

polypeptide that has been inactivated by truncation of a small portion of its carboxyl-terminal region. Neither the specification nor the prior art of record suggests that either the native enzymatic or the native binding activity of polypeptides having appreciable tertiary structure could be restored by ligation of a small, recombinantly-expressed, portion of an "inactive" amino-terminal region of a polypeptide to a synthetic polypeptide comprising the remaining, activating, carboxyl-proximal structure of the polypeptide, or by ligation of roughly equal halves of a polypeptide where the "inactive" amino-terminal portion is recombinantly expressed and the remaining, activating, carboxyl-terminal portion is synthetically produced. Indeed, the only restorations of enzymatic activity in the specification and prior art of record achieved in a manner analogous to that set forth in claim 28 have occurred where, (1) the tertiary structure of the "inactive", or first, polypeptide has already formed properly upon its recombinant expression in a host cell, and (2) the tertiary structure of the "inactive", or first, polypeptide yields an exposed site for ligation of a second, activating, carboxyl-proximal peptide. There is no disclosure or teaching that a carboxyl-proximal, activating, peptide comprising more than 100 amino acids may be ligated to an inactive first, or amino-proximal, region of a polypeptide and the specification and the prior art do not suggest how a claimed ligation method can permit the proper folding of the integral tertiary structures of polypeptides generally. At best, the teachings of the specification and the prior art of record support the ligation of a synthetic peptide to a carboxyl-terminal thioester present in a polypeptide that has already properly folded where an artisan would require no undue experimentation to determine where to truncate the carboxyl terminus of the native polypeptide so that a carboxyl-terminal thioester is available for ligation to a synthetic peptide, but there is no teaching of how to assess whether or not any restoration of an enzymatic or binding activity would result, i.e., the truncation leaving a carboxyl-terminal thioester available for ligation to a synthetic peptide need not inactivate the polypeptide. Applying the

"*Forman*" factors discussed in *Wands*, ***supra***, to Applicant's disclosure, it is apparent that:

> a) the specification lacks adequate, specific, guidance for dividing the primary structures, the amino acid sequences, of proteins into carboxyl-proximal regions that will be inactive, yet fold into their native tertiary structures and have a carboxyl-terminal thioester available for ligation to a synthetic peptide, that might "activate" it,
>
> b) the specification lacks working examples wherein the amino acid sequence of a protein is divided into a carboxyl-proximal region that is inactive yet folds into its native tertiary structures and provides a carboxyl-terminal thioester for ligation to a synthetic protein of equivalent mass that might "activate" it,
>
> c) in view of the prior art publications of record herein, the state of the art and level of skill in the art do not support the practice of a claimed method unless an "active" polypeptide can be truncated to that a synthetic peptide would restore its enzymatic activity upon ligation, and,
>
> d) unpredictability exists in the art where no one has nearly evenly divided the primary structure, the amino acid sequence, of a protein into a carboxyl-proximal region that will be inactive, yet fold into its native tertiary structure, and also have a carboxyl-terminal thioester available for ligation to a synthetic peptide or protein, that might restore its activity.

Thus the scope of practice of methods of claims 28-30 wherein synthetic proteins are ligated to an inactive, recombinantly-expressed, protein to restore a non-specific "protein activity" cannot be considered to be supported by the present specification even if taken in combination with teachings available in the prior art.

The following is a quotation of the second paragraph of 35 U.S.C. §112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 12-18 and 22-30 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 12-16 are indefinite because clause (a) of claim 12 recites, "the precursor protein comprising the protein fused to an intein and optionally a protein binding domain" yet this phrase fails to indicate which is the protein fused to an intein, fails to describe the orientation of the fusion between the unspecified protein and the intein, and fails to describe the relationship of the further protein binding domain to the intein. Claims 12-16 are further indefinite because the last line of claim 12 recites, "so as to

form the target protein having the C-terminal thioester" where no "target protein" has previously been identified in the preceding clauses or the preamble of the claim. Claims 13-16 are included in this rejection because they do not otherwise resolve the ambiguity of claim 12 from which they depend. This aspect of the rejection may be overcome by amending claim 12 to state, e.g.,

"A method for preparing a **selected** protein with a C-terminal thioester, comprising:

(a) expressing in a host cell a recombinant precursor protein comprising the **selected** protein fused **at its carboxyl terminus** to **the amino terminus of** an intein, wherein the **carboxyl terminus of the** intein is optionally fused to a protein binding domain, wherein the intein is capable of being cleaved from the **selected** protein in the presence of 2-mercaptoethanesulfonic acid and is selected from a **modified** intein, an intein derivative, or a mutant intein; and,

(b) contacting the expressed precursor protein with 2-mercaptoethanesulfonic acid and inducing cleavage of the intein from the precursor protein so as to form the **selected** protein having the C-terminal thioester."

Claim 13 is independently indefinite in reciting abbreviations for the genus and species designations of the sources for the two inteins where the abbreviations are susceptible of other interpretations. This aspect of the rejection may be overcome by amending the claim to recite: "The method according to claim 12 wherein the intein is selected from a modified *Saccharomyces cerevisiae* VMA intein and a modified *Mycobacterium xenopi* GyrA intein. Claim 15 independently indefinite where it recites, "wherein the protein is selected from . . ." because it fails to identify which protein of claim 12 that Applicant intends, and is also indefinite in reciting an abbreviation for the genus and species designation of the source for the DNA polymerase fragment which is the first possible selection. Amending claim 15 to recite, in pertinent part, "wherein the

selected protein is selected from a *Bacillus stearothermophilus* DNA polymerase I large fragment", will overcome this aspect of the rejection. Claim 16 is independently indefinite in reciting, "wherein the protein is selected from a maltose binding protein and paramyosin", because no particular protein of claim 12, from which the claim depends, is indicated. Amending claim 16 to recite, "wherein the protein **binding domain** is selected from a maltose binding protein and paramyosin", will overcome this aspect of the rejection.

Claims 17, 18 and 21 are indefinite because, like claim 12, claim 17 fails to indicate the orientation of the components of the fusion gene encoded within the nucleic acid sequence of the acceptor plasmid, and because clause (a)(ii) of claim 17 recites, "the multiple cloning site contains a linker and the linker sequence is selected from", thus ambiguously suggesting that a linker may be other than a nucleic acid sequence, which is intended, and also because clause (b) of claim 17 recites an incomplete process since merely "introducing the plasmid into a host cell" cannot ensure the expression required by the preamble of the claim. Claim 18 is included in this rejection because it fails to resolve the ambiguities of claim 17 from which it depends. This aspect of the rejection may be overcome by amending claim 17 to recite, e.g.,

"A method for expressing a recombinant precursor protein comprising,

(a) inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of, and in frame with, a fusion gene encoding an amino-proximal intein and a carboxyl-proximal binding protein domain wherein,

(i) the intein is selected from a modified intein, an intein derivative, or a mutant intein; and,

(ii) the multiple cloning site comprises a linker nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4; and,

(b) introducing the plasmid into a host cell and providing conditions suitable for the recombinant expression of the recombinant precursor protein by the host cell."

Claim 18 is independently indefinite in reciting, "wherein the binding protein encoded by the nucleic acid sequence" because there is no previous identification of an independent "binding protein encoded by an unspecified nucleic acid sequence in claim 17 from claim 18 depends. This aspect of the rejection may be overcome by amending claim 18 to recite, in pertinent part, "wherein the binding protein domain encoded by the nucleic acid sequence **of the fusion gene** is a chitin binding domain".

Claims 22-24 are indefinite because claim 22, like claim 12 ambiguously recites, in clause (a), "the protein fused to . . . an intein" because it fails to describe the orientation of the fusion between the protein to be modified and the intein. Claims 23 and 24 are included in this rejection because they fail to resolve the ambiguities of claim 22 from which they depend. This aspect of the rejection may be overcome by amending clause (a) of claim 22 to recite, "expressing in a host cell the protein fused **at its carboxyl terminus** to an intein capable of thiol induced cleavage selected from a modified intein, an intein derivative, or a mutant intein". Claim 23 is independently indefinite in reciting, "wherein the protein prior to modification is", because there is no antecedent basis for the term "modification" in claim 22 from which claim 23 depends. One way to overcome this aspect of the rejection is to recite, "a first protein", in the preamble and clauses (a), (b) and (d) of claim 22, allowing claim 23 to recite, "wherein the first protein is". Claim 24 is independently indefinite in reciting, "wherein the intein . . . is optionally fused to a protein binding domain" because indicating an optional embodiment in the dependent claim 24 improperly permits it to exceed the scope of claim 22 from which it depends and also because, as discussed above with respect to claim 12, claim 24 fails to describe the relationship of the further protein binding domain to the intein. Amending

clause (a) of claim 22 to recite, "wherein the intein is optionally fused at its carboxyl terminus to a protein binding domain" will overcome this aspect of the rejection.

Claims 25-27 are indefinite because claim 25 recites, in clause (a), "the precursor protein comprising the target protein fused to an intein and optionally a protein binding domain" because this phrase fails to describe the orientation of the fusion between the target protein and the intein and also fails to describe the relationship of the further protein binding domain to the intein. Claims 26 and 27 are included in this rejection because they fail to resolve the ambiguities of claim 25 from which they depend. Amending clause (a) of claim 25 in the fashion suggested above for an amendment to clause (a) of claim 12 is the best way to address this aspect of the rejection. Claim 27 is independently indefinite in reciting "peptide fragment" because the term "fragment" finds no antecedent basis in claim 25. Amending claim 27 to recite, e.g., "wherein the synthetic peptide or protein comprises an antigenic determinant".

Claims 28-30 are indefinite because claim 28 recites, in clause (a), "expressing in a host cell, a fusion protein comprising the first target protein fused at the C-terminus to an intein . . . " because this phrase fails to relate a "first target protein" to the claim preamble, where this term has been deleted, and because the clause fails to describe the orientation of the fusion between the target protein and the intein. Claim 28 is further rejected as indefinite because clause (b) of the claim recites, "inducing intein mediated cleavage of the protein . . . so as to form a C-terminal thioester on the protein", thus confounding two separate polypeptides, the first of which is the fusion protein – because Applicant does not intend cleavage of the "inactive" or "target" protein – and the second of which is the "inactive" or "target" protein because Applicant intends that the "inactive" or "target" protein has a C-terminal thioester after the cleavage so that it can be ligated to the protein or peptide component of clause (c) of the claim according to clause (d) of the claim. Claim 28 is additionally rejected as indefinite in reciting each

of the terms, "inactive protein", "protein activity", and "inactive form of the protein" because the public cannot ascertain the nature of the activity to be restored. Claims 29 and 30 are included in this rejection because they fail to resolve the ambiguities of claim 28 from which they depend where they do not specify which is the protein of claim 28 that is intended.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 22-25 and 27-30 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 73 of copending Application No. 09/249,543. Claim 73 of the copending application recites, "[a] method for ligating a first and a second target protein comprising (a) combining in a mixture (i) a first target protein having a C-terminus . . . compris[ing] a thioester formed by cleavage of a first intein or modification thereof from a first fusion protein . . . and (ii) a second target protein having an N-terminus, wherein the N-terminus is a cysteine or selenocysteine amino acid, the N-terminus cysteine or selenocysteine resulting from induced cleavage of a second intein or modification thereof in a second fusion protein . . . and (b) ligating the first and second target proteins" (underlined for emphasis).

Although the conflicting claims are not identical, they are not patentably distinct from each other where claim 73 of the copending application embraces ligation methods of

claims 22-25 and 27-30 herein because (1) both applications disclose the induction of cleavage of a first protein fused to an intein present at the carboxyl-proximal junction of the first protein with 2-mercaptoethanesulfonic acid, (2) clause (a)(ii) of claim 73 of the copending application does not require that a second target protein be cleaved from a recombinantly-expressed, precursor, fusion protein, and (3) recitation "fusion protein" in clause (a)(ii) of claim 73 is not a distinguishing limitation where the term may be applied to any protein, whether recombinantly-expressed or made by solid phase synthesis, to merely indicate that the amino acid sequence of a polypeptide comprises components of two or more different polypeptides. A patent issued with claim 73 of the copending application would embrace ligation methods of claims 22-25 and 27-30 herein since methods of making final products using first proteins of clauses (a) and (b) of claim 22, clauses (a) and (b) of claim 25, or clauses (a) and (b) of claim 28, by ligating them in a process of step (c) of claims 22, 25 and 28 to another peptide or protein of clause (d) of these claims, and their dependent claims, is a method covered by claim 73 of the copending application regardless of the nature of the ligation partners of clauses (d) of claims 22, 25 and 28 herein, just as a generic method of ligating a first protein of clause (a)(i) of claim 73 with any component having an amino acid sequence is the practice of claims 22-25 and 27-30 herein. Patenting of pending claims in both applications would thus constitute an unjustified or improper timewise extension of the "right to exclude" granted by a patent and it would be obvious to one of ordinary skill in the art that the various final products resulting from methods of claims 22-25 and 27-30 herein are also products made by the generic method of claim 73 of the copending application where clause (a)(ii) does not affirmatively require that second ligation partner be recombinantly expressed. This is a provisional obviousness-type double patenting rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. §103(c) and potential §§35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. §103(a).

Claims 12-14, 22, and 24-26 are rejected under 35 U.S.C. §103(a) as being anticipated by Chong et al., 1997, **Gene**, Vol. 192, pages 271-281, of record, and Burton et al., U.S. Patent No. 5,789,578, in view of the description of 2-mercaptoethanesulfonic acid - MESNA - in the Dictionary of Organic Compounds, Chapman & Hall, New York, Sixth Edition, 1996, Volume Four, at page 4155, cited above.

Chong et al. teach methods of preparing a first protein having a C-terminal thioester upon thiol reagent-induced cleavage of a modified *Saccharomyces cerevisiae* VMA intein fused at its amino terminus to the carboxyl terminus of the first protein wherein the modified intein is further fused at its carboxyl terminus to a chitin binding protein, analogous to methods of claims 12-14 herein, save for the use of the specific thiol reagent. **See**, Figures 1, 4 and 5B, and section 2.6 at page 276. Chong et al. teach that free cysteine, β-mercaptoethanol, or dithiothreitol [DTT], should be used to produce a C-terminal thioester rather than MESNA. Chong et al. further teach methods of preparing a labeled first protein by ligating a radiolabeled peptide comprising an amino terminal cysteine to the C-terminal thioester of the cleaved, thioester-bearing, first protein, analogous to methods of claims 22 and 24-26 herein.

Burton et al. teach that any of several thiol reagents, including MESNA, will provide a reactive thiol ligand for the purpose of ligating a target compound such as a protein or

a peptide. **See**, paragraph spanning cols. 5 and 6 and col. 6, lines 41 and 42. The Dictionary of Organic Compounds teaches that the pKa of the thiol group of MESNA is 9.53 and one of ordinary skill in the art at the time the invention was made would have readily appreciated that MESNA, a low molecular weight acid, is freely soluble in water. It would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute MESNA for β-mercaptoethanol, which has a pKa of 9.71, or free cysteine in the methods of Chong et al. because MESNA has much greater solubility than free cysteine or β-mercaptoethanol in aqueous solution and a more electronegative pKa than that of β-mercaptoethanol according to The Dictionary of Organic Compounds, and because it provides a suitably reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al. Such an artisan would have been motivated to substitute MESNA for the thiol reagents used in the methods of Chong et al. in view of its greater solubility than free cysteine or β-mercaptoethanol in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al. .

Claims 12-14, 22, 24-26, and 28 are rejected under 35 U.S.C. §103(a) as being anticipated by Severinov et al., 1998, **The Journal of Biological Chemistry**, Vol. 273, pages 16205-16209, of record, and Burton et al., U.S. Patent No. 5,789,578, in view of the description of 2-mercaptoethanesulfonic acid - MESNA - in the Dictionary of Organic Compounds, Chapman & Hall, New York, Sixth Edition, 1996, Volume Four, at page 4155, cited above.

Severinov et al. teach methods of preparing a first expressed protein, a portion of the *E. coli* RNA polymerase σ^{70} subunit, having a C-terminal thioester upon thiol reagent-induced cleavage of a modified *Saccharomyces cerevisiae* VMA intein fused at its amino terminus to the carboxyl terminus of this first protein wherein the modified intein is further fused at its carboxyl terminus to a chitin binding protein, analogous to methods of claims 12-14 herein, see pages 16205-09 and Table 1. Severinov et al., however, teach the use of thiophenol, mercaptoacetic acid, free cysteine, or dithiothreitol, rather than MESNA, see page 16206. Severinov et al. further teach

methods of labeling a target protein by ligating a synthetic peptide that comprises both a fluorescent marker and an amino-terminal cysteine to a portion of the *E. coli* RNA polymerase σ^{70} subunit having a C-terminal thioester resulting from thiol reagent-induced cleavage of a modified *Saccharomyces cerevisiae* VMA intein fused at its amino terminus to the carboxyl terminus of their first protein wherein the modified intein is further fused at its carboxyl terminus to a chitin binding protein, analogous to methods of claims 25 and 26 herein, see Figure 1. Severinov et al. however teach the use of thiophenol to produce a C-terminal thioester, rather than MESNA, see page 16207. Severinov et al. also teach methods of preparing an active *E. coli* RNA polymerase σ^{70} subunit by ligating a synthetic peptide having an amino-terminal cysteine and the carboxyl-terminal 34 amino acids of the polymerase σ^{70} subunit to the C-terminal thioester of the thiol-reagent cleaved, truncated and inactive, *E. coli* RNA polymerase σ^{70} subunit, restoring its activity, analogous to methods of claims 22, 24, and 28 herein save for their use of thiophenol to produce a C-terminal thioester rather than MESNA. **See**, Figure 2 and discussion at pages16208 and 16209,

Burton et al. teach that any of several thiol reagents, including MESNA, will provide a reactive thiol ligand for the purpose of ligating a target compound such as a protein or a peptide. **See**, paragraph spanning cols. 5 and 6 and col. 6, lines 41 and 42. The Dictionary of Organic Compounds teaches that the pKa of the thiol group of MESNA is 9.53 and one of ordinary skill in the art at the time the invention was made would have readily appreciated that MESNA, a low molecular weight acid, is freely soluble in water. It would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute MESNA for the thiol reagents used in the methods of Severinov et al. because MESNA has much greater solubility than thiophenol or free cysteine in aqueous solution and, according to The Dictionary of Organic Compounds, a more electronegative pKa thus providing a suitably reactive thiol ligand for the purpose of

ligating a protein or peptide according to Burton et al. Such an artisan would have been motivated to substitute MESNA for the thiol reagents used in the methods of Severinov et al. in view of its solubility in aqueous solution and more electronegative pKa and because it provides a suitably reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al.

Claims 12-14, 22, 24 and 28 are rejected under 35 U.S.C. §103(a) as being obvious over Muir et al., 1998, of record, and Burton et al., U.S. Patent No. 5,789,578, cited above, in view of the description of 2-mercaptoethanesulfonic acid - MESNA - in the Dictionary of Organic Compounds, Chapman & Hall, New York, Sixth Edition, 1996, Volume Four, at page 4155, cited above.

Muir et al. teach, pages 6706-09 and Figures 1-4, a method of preparing an protein, an inactive protein tyrosine kinase, that comprises a C-terminal thioester produced by thiol reagent-induced cleavage of a modified *Saccharomyces cerevisiae* VMA intein fused to the carboxyl terminus of the kinase wherein the modified intein is also fused at its carboxyl terminus to a chitin binding protein, permitting purification of the thioester-comprising kinase inactive with its native substrates, analogous to methods of claims 12-14 herein save for the use of the specific thiol reagent, where Muir et al. teach the use of thiophenol rather than MESNA. Muir et al. also teach methods of preparing a modified, active, kinase by ligating an undecapeptide comprising an amino terminal cysteine to the C-terminal thioester of the cleaved, thioester-bearing, inactive kinase analogous to methods of claim 22 and 24 herein and further teach that ligation of the synthetic undecapeptide, which has a consensus sequence of conserved activating phosphorylation sites of Src kinases and an amino-terminal cysteine, to the inactive kinase having a C-terminal thioester upon thiol reagent-induced cleavage of the modified *Saccharomyces cerevisiae* VMA intein fused to the carboxyl terminus of the kinase greatly augments its activity, analogous to a method of claim 28 herein, save for the use of the specific thiol reagent where Muir et al. teach the use of thiophenol to produce a C-terminal thioester rather than MESNA.

Burton et al. teach that any of several thiol reagents, including MESNA, will provide a reactive thiol ligand for the purpose of ligating a target compound such as a protein or a peptide. **See**, paragraph spanning cols. 5 and 6 and col. 6, lines 41 and 42. The Dictionary of Organic Compounds teaches that the pKa of the thiol group of MESNA is 9.53 and one of ordinary skill in the art at the time the invention was made would have readily appreciated that MESNA, a low molecular weight acid, is freely soluble in water. It would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute MESNA for thiophenol in the methods of Muir et al. because MESNA has much greater solubility than thiophenol in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al. Such an artisan would have been motivated to substitute MESNA for thiophenol in the methods of Muir et al. in view of its much greater solubility than thiophenol in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al.

Claims 15, 16, 23, 27, 29 and 30 are rejected under 35 U.S.C. §103(a) as obvious over Chong et al., 1997, as applied to claim 12 above and Severinov et al., as applied to claims 12, 22, 24 and 28 above, and further in view of Comb et al., U.S. Patent No. 5,834,247, of record, Burton et al., U.S. Patent No. 5,789,578, cited above, and the description of 2-mercaptoethanesulfonic acid - MESNA - in the Dictionary of Organic Compounds, Chapman & Hall, New York, Sixth Edition, 1996, Volume Four, at page 4155, cited above.

The teachings of Chong et al., 1997, are taken as before, particularly the method of preparing a target protein fused at its carboxyl terminus to a modified *Saccharomyces cerevisiae* VMA intein which is itself fused at its carboxyl terminus to the chitin binding protein, where the target proteins include several restriction endonucleases, enzymes which one of ordinary skill in the art would have been well aware to be inherently cytotoxic to any host cell in which they are expressed in the absence of expression of a corresponding, protective, methylase, which protection would permit survival of the host and isolation of a recombinantly-expressed restriction endonuclease. See Table 1 at

page 277. These teachings are further combined with the teaching of Chong et al. that "thiol esters that result from intein-mediated cleavage induced by thiol compounds can serve as intermediates in peptide ligation", see page 279, left column of text, and combined as well with the teachings of Severinov et al., discussed above, of the division of the amino acid sequence of an enzyme into an inactive amino-proximal region that is recombinantly expressed as a fusion polypeptide comprising a carboxyl-proximal, modified, intein, whereby the inactive amino-proximal region subsequently acquires a C-terminal thioester upon cleavage of the modified intein with a thiol reagent, as well as a carboxyl-proximal region prepared by solid-phase synthesis that represents the rest of its amino acid sequence and comprises an amino-terminal cysteine, to permit ligation of the two regions mediated by the C-terminal thioester of the amino-proximal portion and the concomitant restoration of enzymatic activity.

Comb et al. teach a method of making a labeled expressed protein, which is also a modified expressed protein, by ligating labeled synthetic peptides having amino terminal cysteines to the C-terminal thioester of the expressed maltose binding protein, wherein the labels are peptides having amino-terminal cysteines, some of which are antigenic determinants, analogous to methods of claims 16 and 27 herein. See Figure 29. Comb et al. further teach that "[t]his method can be utilized to synthesize as functional proteins such as enzymes that are toxic to host cells", see col. 77 at lines 52-54. It would have been obvious to one of ordinary skill in the art at the time the invention was made to recombinantly express an inactive, truncated, restriction endonuclease as the amino-proximal region of a fusion polypeptide comprising a modified *Saccharomyces cerevisiae* VMA intein fused at its carboxyl terminus and to then generate an inactive, truncated, restriction endonuclease comprising a C-terminal thioester upon cleavage of the modified intein with a thiol reagent according to the teaching of Chong et al. and the suggestions of Comb et al. and in the fashion taught by Severinov et al., as well as to

prepare a synthetic peptide comprising the remaining amino acid sequence of the restriction endonuclease with an amino terminal cysteine and to then ligate the synthetic peptide *in vitro* to the expressed inactive, truncated, restriction endonuclease comprising a C-terminal thioester to restore its enzymatic activity according to the teachings of Severinov et al. This is because Chong produced restriction endonuclease in low yields with a method similar to, and a plasmid similar to, that of Comb et al. due to the toxicity of these enzymes to the host cells, because Comb et al. suggest that their method should be used to produce toxic proteins, because Chong et al. acknowledge that "thiol esters that result from intein-mediated cleavage induced by thiol compounds can serve as intermediates in peptide ligation", and because Severinov et al. show that such suggestions are predictable and efficacious by demonstrating how to divide the amino acid sequence of an enzyme into a large, inactive, amino-proximal region for recombinant expression fused to a modified intein at its carboxyl-terminus as well as how to generate a C-terminal thioester upon cleavage of the modified intein with a thiol reagent in order to ligate the inactive amino-proximal region of the enzyme to an activating, carboxyl-proximal, peptide prepared by solid-phase synthesis and comprising an amino-terminal cysteine to restore enzymatic activity.

Burton et al. teach, that MESNA and several other thiol reagents will provide a reactive thiol ligand for the purpose of ligating a target compound such as a protein or a peptide. **See**, paragraph spanning cols. 5 and 6 and col. 6, lines 41 and 42. The Dictionary of Organic Compounds teaches that the pKa of the thiol group of MESNA is 9.53 and one of ordinary skill in the art at the time the invention was made would have readily appreciated that MESNA, comprising both a thiol and a sulfonic acid, is freely soluble in water. It would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute MESNA for thiol reagents used in methods of Chong et al. and Severinov et al. because MESNA has according to The Dictionary of

Organic Compounds, and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al. Such an artisan would have been motivated to substitute MESNA for thiol reagents used in a method of Chong et al. as modified by teachings of Severinov et al. and Comb et al. for making a cytotoxic protein, such as a restriction endonuclease, in view of its greater solubility than free cysteine or β-mercaptoethanol in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al.

Claim 13 is rejected under 35 U.S.C. §103(a) as being anticipated by Chong et al., 1997, as applied to claim 12 above, in view of Telenti et al., 1997, of record, and further in view of Burton et al., U.S. Patent No. 5,789,578, cited above, and the description of 2-mercaptoethanesulfonic acid in the Dictionary of Organic Compounds, Chapman & Hall, New York, Sixth Edition, 1996, Volume Four, at page 4155, cited above.

The teachings of Chong et al., discussed above, are taken as before. Telenti et al. disclose a modified intein comprising a mutant *Mycobacterium xenopi GyrA* intein that is, see results in Table 1 with the C114R mutant in the "MIEP" expression construct at page 6380, capable of thiol reagent-induced cleavage producing a thioester at the carboxyl-terminus of a polypeptide fused to the intein within a precursor protein. Telenti et al., like Chong et al., use DTT as a thiol reagent to produce a C-terminal thioester rather than MESNA. It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the modified *Mycobacterium xenopi* GyrA intein of Telenti et al. for the modified *Saccharomyces cerevisiae* VMA of Chong et al. in practicing a method of claim 13 because Telenti et al. demonstrate to such an artisan at that time that their modified *Mycobacterium xenopi* GyrA intein is capable of producing a thioester at the C-terminus of a polypeptide fused to an intein within a precursor protein when contacted with a thiol reagent to cleave the intein, thus is suitable in a method of claims 12 and 13.

Burton et al. teach, see paragraph spanning cols. 5 and 6, that either DTT or MESNA will provide a reactive thiol ligand for the purpose of ligating a target compound such as, col. 6, lines 41 and 42, a protein or a peptide. The Dictionary of Organic Compounds teaches that the pKa of the thiol group of MESNA is 9.53, intermediate between the pKas of the thiol groups of DTT, and one of ordinary skill in the art at the time the invention was made would have readily appreciated that MESNA, a low molecular weight acid, is freely soluble in water. It would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute MESNA for the thiol reagents used in a method of Chong et al. modified by use of the mutant intein of Telenti et al. because MESNA is completely soluble in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al. Such an artisan would have been motivated to substitute MESNA for the thiol reagents used in a method of Chong et al. modified by use of the mutant intein of Telenti et al. because it has better solubility than DTT in aqueous solution and because it provides a suitable reactive thiol ligand for the purpose of ligating a protein or peptide according to Burton et al.

Conclusion

Claim 21 is objected to as being dependent upon a rejected base claim but is free of the prior art of record herein and, although rejected above under the first and second paragraphs of 35 U.S.C. §112, claims 17 and 18 are also free of the prior art of record in view of the amendment to claim 17.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 703.308.0583 until about January 21, 2004, and will be 571.272.0933 thereafter. The examiner can normally be reached between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at 703.308.3804 until about January 21, 2004, and at 571.272.0928 thereafter. The fax phone numbers for all communications for the organization where this application or proceeding is assigned is 703.872.9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.0196.

5 William W. Moore
November 21, 2003

NASHAAT T. NASHED PHD.
PRIMARY EXAMINER